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The Identification and Characterization of Osmotolerant Yeast Isolates from Chemical Wastewater Evaporation Ponds

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ABSTRACT

Ramat Hovav is a major chemical industrial park manufacturing pharmaceuticals, pesticides, and various aliphatic and aromatic halogens. All wastewater streams are collected in large evaporation ponds. Salinity in the evaporation ponds fluctuates between 3% (w/v) and saturation and pH values range between 2.0 and 10.0. We looked for microorganisms surviving in these extreme environmental conditions and found that 2 yeast strains dominate this biotope. 18S rDNA sequence analysis identified the isolates as *Pichia guilliermondii* and *Rhodotorula mucilaginosa*. Both isolates grew in NaCl concentrations ranging up to 3.5 M and 2.5 M, respectively, and at a pH range of 2–10. There was a distinct difference between the *Rhodotorula* and *Pichia* strains and *S. cerevisiae* RS16 that served as a control strain with respect to accumulation of osmoregulators and internal ion concentrations when exposed to osmotic stress. The *Pichia* and *Rhodotorula* strains maintained high glycerol concentration also in media low in NaCl. Utilization of various carbon sources was examined. Using a tetrazolium-based assay we show that the *Rhodotorula* and *Pichia* strains are capable of utilizing a wide range of different carbon sources including anthracene, phenanthrene, and other cyclic aromatic hydrocarbons.

Introduction

The Ramat Hovav industrial park, located 12 km south of Beer Sheba in Israel's southern Negev Desert, is a major manufacturing site for various pesticides, pharmaceuticals, and aliphatic and aromatic halogens. All wastewater generated throughout the manufacturing processes is collected in large evaporation ponds. Salinity in these ponds fluctuate between 3% (w/v) and saturation while pH values vary between 2.0 and 10.0. Organic matter concentration is 5–10 g/L [3]. We looked for microorganisms in this biotope and found that it is dominated by two yeast strains, *Rhodotorula mucilaginosa* (*R. mucilaginosa*) and *Pichia guilliermondii* (*P. guilliermondii*).

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Many yeast and other fungi are well suited for life in extreme environments with respect to high osmotic pressures, acidic pH [20], and ability to grow in polluted environments [32]. To date, only a few studies have been carried on the biodegradative potential of yeast in aquatic environments [14].

Yeast versatility in utilizing various carbon sources hints at their participation in biodegradative processes that might be of significance in the removal of toxic organics from the stored wastewater during the evaporation process. In earlier work it was found that the number of yeast cells in polluted waters increases with the increase in contaminant concentration, so much so that the number of red-pigmented yeast cells such as *Rhodotorula* sp. was proposed as an index of pollution in contaminated waters [32]. The range of carbon sources known to be metabolized or cometabolized by yeast strains is very wide. For example, it was shown that in the presence of glucose, pure cultures of *Saccharomyces cerevisiae* and *Candida utilis* co-oxidize naphthalene and anthracene [17]; *Candida guilliermondii, Debaryomyces hansenii, Candida tropicales,* and *Candida maltosa* oxidize naphthalene and benzo[α]pyrene [11].

Considerable effort was put into the study and understanding of mechanisms involved in salt tolerance of yeast. The moderately halotolerant *S. cerevisiae* was studied extensively [1, 5, 6, 9, 31] as well as the extremely halotolerant yeast strains *D. hansenii* [25, 29], *Pichia sorbitophila* [24], and *Zygosaccharomyces rouxii* [32].

In this study we characterized the physiological responses of the yeast strains that were isolated from the evaporation ponds and looked at a variability of carbon sources available for these isolates.

Materials and Methods

Yeast Strains and Growth

Ramon Serrano (Spain) graciously donated *S. cerevisiae* strain RS16 [12] (*MAT*a, *leu2-3, 112, ura3-251, 328, 372).* All other yeast strains were isolated from the chemical industry wastewater evaporation ponds. Samples from the evaporation ponds were analyzed for pH and Na^+ content and the ponds exhibiting the most extreme conditions were chosen for further work. Water samples were filtered and the concentrate was plated on agar plates made with pond water. Colonies of yeast exhibiting extensive growth were selected for further studies. The isolated strains were grown routinely on YPD medium containing 1% yeast extract, 2% Bacto-Peptone, and 2% glucose. For growth experiments YPD medium or 0.67% yeast nitrogen base without amino acids (YNB) medium was used. YNB (10× strength) was filter sterilized, while glucose and salt solutions were autoclaved separately. NaCl was added as indicated. In order to examine the effect of pH on yeast growth, the medium was prepared in either of the following buffer systems: 0.1 M citric acid–0.2 M sodium dihydrogen phosphate (pH 2.0–8.0); or 0.1 M potassium dihydrogen phosphate–0.1 M NaOH (pH 6.0–10.0).

Carbon source utilization experiments were carried out with cells grown on mineral salts medium (MM) containing (per liter) 5 g $(NH_4)H_2PO_4$, 2.5 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 2 mg $FeCl_3 \cdot 6H_2O$, 20 mg $Ca(NO_3)_2 \cdot 4H_2O$, and 10 mL of a trace element solution (containing, per liter, 50 mg H_3BO_3, 40 mg $MgSO_4 \cdot 4H_2O$, 40 mg $ZnSO_4 \cdot 7H_2O$, 20 mg Na_2MoO_4, 10 mg $CuSO_4 \cdot 5H_2O$, 10 mg $CoCl_2$, 10 mg potassium iodide).

Growth was followed by observing the culture's absorbance at 660 nm with a Hewlett Packard 8452A diode array spectrophotometer. Growth as a function of absorbance was calibrated by cell counts using a Neubauer hemocytometer. Cultures were grown at 25°C and shaken on a rotary shaker (New Brunswick Scientific, Innova 2300 platform shaker) at 150–180 rpm.

Drop Test Assay

The drop test assay, as previously described [4], was used in order to characterize the growth of the two yeast isolates and the *S. cerevisiae* control strain at different combinations of pH/NaCl (buffers and growth conditions given above) and different concentrations of external solutes. Colony forming capacity on different external solute concentrations was determined using increasing concentrations of LiCl, NaCl, KCl, and sorbitol.

Characterization and Classification of the Yeast Isolates

After amplification and sequencing of the gene encoding for the ribosomal small subunit [19] 18S rRNA, the data were compared to sequences available in known databases. Amplification of the 5′ end (ca. 1300 to 1400 bp) of the gene encoding for 18S rRNA was obtained by using the primer combination P108/M3490:

P108 5′: ACCTGGTTGATCCTGCCAGT (positions 2-21 of *S. cerevisiae*). M3490 5′ TCAGTGTAGCGCGCGTGCGG (ca. 1473–1454). For species identification the following primers were used: P1190 5′ CAATTGGAGGGCAAGTCTGG (ca. 543-562) and M2130 5′ CAATAAATCCAAGAATTTCACC (ca. 900–921).

Determination of Intracellular Na^+ and K^+ Content

Samples were prepared as previously described [25]. Of the cultures grown in YPD medium containing high NaCl (2 M for the *Rhodotorula* and *Pichia* strains and 1 M for the *S. cerevisiae* strain) or high LiCl, 1.5 mL was filtered through a 0.45 μm filter (Millipore ME 25, prewashed with iso-osmotic $CaCl_2$) and washed five times with 3 mL of iso-osmotic $CaCl_2$. The filter was then immersed in 3 mL of distilled water, heat treated at 95°C in

tubes with a pear drop condenser for 10 min, and centrifuged for 5 min at 3500 × *g*. The supernatants were kept frozen at −20°C until analysis. Measurements of K^+ and Na^+ content were performed by an atomic absorption spectrometer (Perkin-Elmer Corp, 1100B) in flame emission mode. Washed filters without cells gave similar background values irrespective of medium salinity that were subtracted from all data.

Intracellular Volume Determination

The cellular volume was estimated by measuring cellular size under different salinities by optical microscopy using a micrometer as previously described [16]. Where relevant, results were compared to known cell volumes and found to be consistent.

^{1}H-NMR Analysis of Intracellular Metabolites

Cells (*R. mucilaginosa, P. guilliermondii,* and *S. cerevisiae*) were grown to mid-exponential phase of growth in absence or presence of NaCl (2 M for both the *Rhodotorula* and *Pichia* strains, and 1 M for the *Saccharomyces* strain), spun (7500 rpm, 10 min, 20°C), washed with cold distilled water, and then lyophilized (Virtis Co., 10-MR-TR lyophilizer). One gram of lyophilized cells was resuspended in water and disrupted by passing through a French pressure cell (SLM-Aminco) at 3.3 MPa four times. Small aliquots of each sample (200 μL) were boiled for 5 min and protein concentrations were determined by the Bradford method [8]. Extraction of intracellular solutes was performed by boiling the French press lysates in 80% ethanol (v/v) for 10 min. The extraction was repeated twice and the combined extracts were evaporated under vacuum. The residue was dissolved in a mixture of water and chloroform (2:1) and centrifuged to remove any lipid components. The aqueous phase was dried in a Speed-Vac (Savant SC100), dissolved in ^1H_2O, and analyzed by ^{1}H-NMR.

^{1}H-NMR spectra were recorded on a Bruker DRX-500 spectrometer and acquired with a 5-mm broad-band probe head, with presaturation of the water signal. Free induction decays were acquired in 32K data points covering a spectral width of 8 kHz, by use of a 45° flip angle and a repetition time of 32 s. The probe head temperature was 25°C. The intensity of the resonance due to a known amount of sodium formate added to the sample was used for quantification. Chemical shifts were referenced with respect to sodium 3-trimethylsilyl[2,2,3,3-^{2}H]propionate.

Determination of Biomass Composition and Low Molecular Weight Metabolites

Biomass analysis was based on a method previously reported [2, 16] using a mixture of methanol: chloroform (2:1). The high molecular weight metabolites (HMWM), containing proteins and polysaccharides, and the low molecular weight metabolites (LMWM), comprising the amino acid, sugars, and glycerol contents in the aqueous phase, were estimated according to Band et al., by standard spectrophotometric techniques.

Carbon Source Utilization Experiments

Potential carbon source utilization was measured by quantifying respiration of prestarved cells in the presence of different carbon sources. Respiration was assayed by the measurement of the reduction of a tetrazolium salt, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), to formazan [18]. Previously starved yeast cells (10^6 cells/mL) were inoculated in 10 mL mineral medium (MM) with a respective carbon source and incubated for 24 h at 30°C. MM stock solution (0.25 mL) containing 5 mg/mL MTT (Sigma M-5655) was added to 1 mL of the above solution and incubated for a further 3 h at 37°C. The cells were then spun down at 1000 × *g* for 10 min and the supernatant was carefully aspirated. One mL of acid isopropanol (95 mL isopropanol acidified by 5 mL 1 N HCl) was added to the cell pellet and shaken for 5 min in order to extract the formazan formed. Absorbance was measured at 550 nm. All assays were performed in duplicates.

The carbon sources tested were toluene, bromobenzene (BB), glucose, 2-fluorophenol (2-FP), benzyl alcohol (BA), bromobenzoic acid (BBA), benzoic acid, bisphenol A (BPA), phenol, naphthalene, phenanthrene, anthracene, methanol, petroleum ether, 1-methylnaphthalene, bromoacetic acid, m-xylene, and raw evaporation pond water. All carbon sources, except for raw wastewater, were tested at concentrations of 100 ppm. Raw wastewater was prepared by diluting 1 mL into 9 mL MM containing 10^6 cells/mL of previously starved cells.

Results and Discussion

Growth and Characterization of the Yeast Isolates

Two yeast strains were isolated from the evaporation ponds using standard isolation techniques (see above). These were the dominant organisms in the pond waters when pH values were left at their original low values of pH 2.6–5 and salinity of 8%–20%w/v. The two isolates were the red-pigmented nonfermentative diploid budding yeast *R. mucilaginosa* and the fermentative haploid yeast *P. guilliermondii* as established by 18S rRNA sequence homology (99.0% and 99.7% identity, respectively; results not shown). *R. mucilaginosa* and *P. guilliermondii* cell ploidy were corroborated to the results found in the literature by quantification of DNA content by fluorescence activated cell sorter analysis (FACS), the former being a naturally occurring diploid while the latter is a naturally occurring haploid yeast (results not shown).

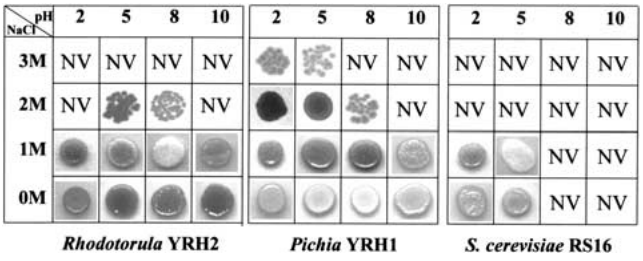

Fig. 1. Drop test assay representing colony formation on a salt/pH matrix of *Rhodotorula* strain YRH2, *Pichia* strain YRH1, and *S. cerevisiae* strain RS16. NV represents no observable colony formation (NV = none visible). The presented results are representative of two independent experiments.

Salt and pH Tolerance

Using the drop test assay [4] the results show that both yeast strains grow within a wide range of salt concentration and pH values (Fig. 1). *P. guilliermondii* grows well at 3 M NaCl and pH 2–5, while *R. mucilaginosa* tolerates the combination of 2 M NaCl and pH 5–8. There is a distinct combined effect of both salt and pH stresses on the growth of both strains (Fig. 1). It has been shown previously that the effect of pH of the medium on growth in the presence of salt is strain dependent [30]. Previously it was shown [16] that in *Yarrowia lipolytica, D. hansenii* and *S. cerevisiae* the highest salt tolerance occurred at pH values of 5.0–7.0, while other yeast strains such as *Kloeokera apiculata, Pichia membranaefaciens,* and *Zygosaccharomyces bailii* exhibited greatest salt tolerance at lower pH values of 3.0–5.0 [30]. Our experimental results suggest that the *Rhodotorula* isolate belongs to the former group of organisms, as they are most tolerant to salt at the pH values of 5–8, whereas the *Pichia* belongs to the group of organisms that are more tolerant to high salt at lower pH values. The biochemical basis for the interactive effect of salt and pH tolerance remains unknown although it might be related to the activity of a plasma membrane ATPase [30].

In rich liquid medium (YPD), *P. guilliermondii* grows at salt concentrations of up to 3.25 M with a negligible effect on final yield. When shifted from low- to high-salt medium there was an increase in the lag period of 4 days compared to growth in the absence of salt (Fig. 2). *R. mucilaginosa* grows at salt concentrations of up to 2.5 M NaCl in accordance with previous work [27], compared to *S. cerevisiae* that was inhibited at salt concentrations of 1.5 M. There is no information on NaCl tolerance of *P. guilliermondii,* although it is a well-known food-spoilage yeast that has been previously isolated from "Ume zuke" (a Japanese apricot product) [28] and forms a distinct phylogenetic group together with the extremely halotolerant yeast *D. hanseniii* [10]. We found that *P. guilliermondii* is indeed much more halotolerant than *R. mucilaginosa.*

The growth of these yeast isolates in the presence of increasing concentrations of LiCl, NaCl, KCl, and sorbitol was determined in a drop-test assay [4] (Fig. 3). *R. mucilaginosa* strain RH2 and *S. cerevisiae* strains formed

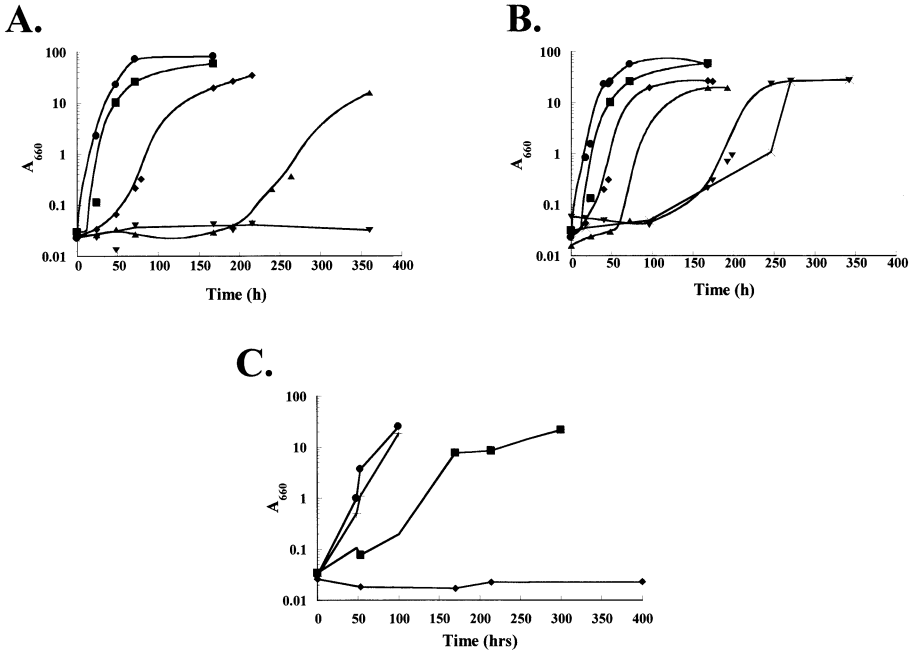

Fig. 2. Growth curves of *Rhodotorula* strain YRH2 (A), *Pichia* strain YRH1 (B), and *S. cerevisiae* strain RS16 (C) on YPD medium supplemented with 0 M (●), 0.5 M (+), 1 M (■), 2 M (♦), 2.5 M (▲), 3M (▼), and 3.5 M (X) NaCl.

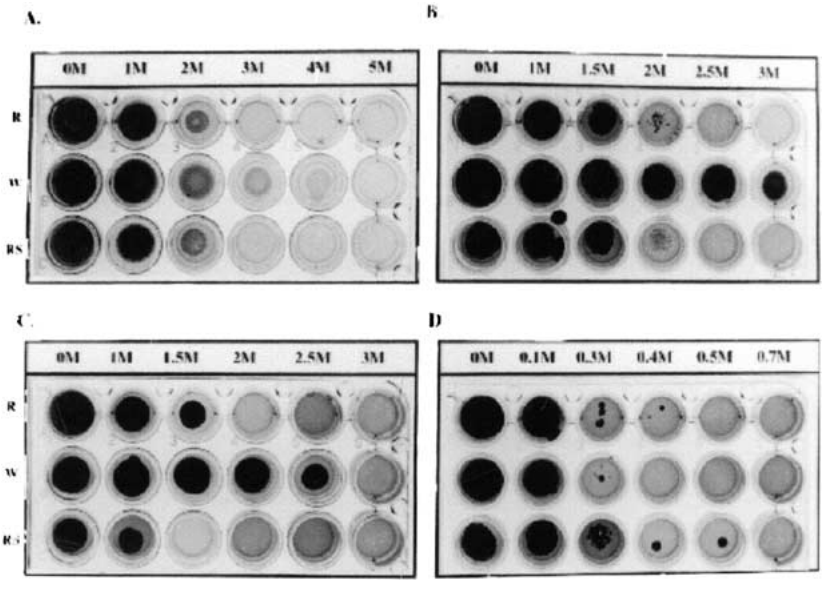

Fig. 3. Drop test assay of *Rhodotorula* strain YRH2 (R), *Pichia* strain YRH1 (W), and *S. cerevisiae* strain RS16 (RS). Cells were grown on YPD medium pH 5, supplemented with increasing concentrations of sorbitol (A), KCl (B), NaCl (C), and LiCl (D). The results are representative of three independent experiments.

colonies on medium containing sorbitol concentrations up to 2 M while *P. guilliermondii* strain RH1 formed colonies at 4 M. It also formed colonies on solid medium containing 3 M KCl and 2.5 M NaCl, while *R. mucilaginosa* did not form colonies at salt concentrations above 1.5 M NaCl and 2 M KCl. *P. guilliermondii* showed higher tolerance to NaCl but exhibited a slightly decreased resistance to 0.3 M LiCl in contrast to both *R. mucilaginosa* and *S. cerevisiae*.

Our observation that Li^+ was slightly more toxic to *P. guilliermondii* strain RH1 suggests that different resistance mechanisms to different cations are involved. *R. mucilaginosa* showed a similar resistance as that of *S. cerevisiae* except for an increased resistance to NaCl.

Internal Ion Concentration

Figure 4 describes the accumulation of salts by the tested strains. It was shown for *S. cerevisiae* that Na^+:K^+ ratios above 0.5 are toxic [13]. The data presented in Fig. 4 show that upon increase in external salts concentration *R. mucilaginosa* and *S. cerevisiae* increased this ratio to 1.1 and 1.3. On the other hand, *P. guilliermondii* maintained a Na^+:K^+ ratio lower than 0.5 during exposure to high external Na^+ concentrations.

Although there was no significant difference in their intracellular Na^+ concentrations, both *R. mucilaginosa* and *P. guilliermondii* had significantly higher internal K^+ concentrations. There was also an initial high internal concentration of K^+ in the unstressed *P. guilliermondii*, possibly providing it with a survival advantage under the varying salt conditions to which it is exposed in the evaporation ponds. Previous work by Prista et al. [29] showed that the toxicity of Na^+ and K^+ in *D. hansenii* is similar. This is also true for *P. guilliermondii* as well, suggesting that for these strains there is no specific toxicity associated with Na^+. However, Wieland et al. [33] presented evidence that the toxic levels of Na^+ are lower than those for K^+ in S. *cerevisiae*.

With regard to Li^+, it was slightly more toxic to *P. guilliermondii* than to *R. mucilaginosa* and *S. cerevisiae* (Fig. 3).

Glycerol, Carbohydrate, Protein, and Amino Acid Accumulation under Salt Stress

After shift from low- to high-salt medium the predominant solute produced was glycerol (Table 1), which is consistent with results from previous studies [6, 7]. In the presence of high salt *S. cerevisiae* started to accumulate glycerol instantaneously, increasing internal glycerol concentration from 18 mM to 658 mM, a 35-fold increase. In *P. guilliermondii* there was an increase of 5.5-fold in internal glycerol following a shift to 2 M NaCl, reaching an internal concentration of 1.4 M glycerol. *R. mucilaginosa* accumulated 1.01 M glycerol after transfer to 2 M NaCl, a 2-fold increase.

Both evaporation pond strains maintain a relatively high concentration of glycerol even in low-osmotic me-

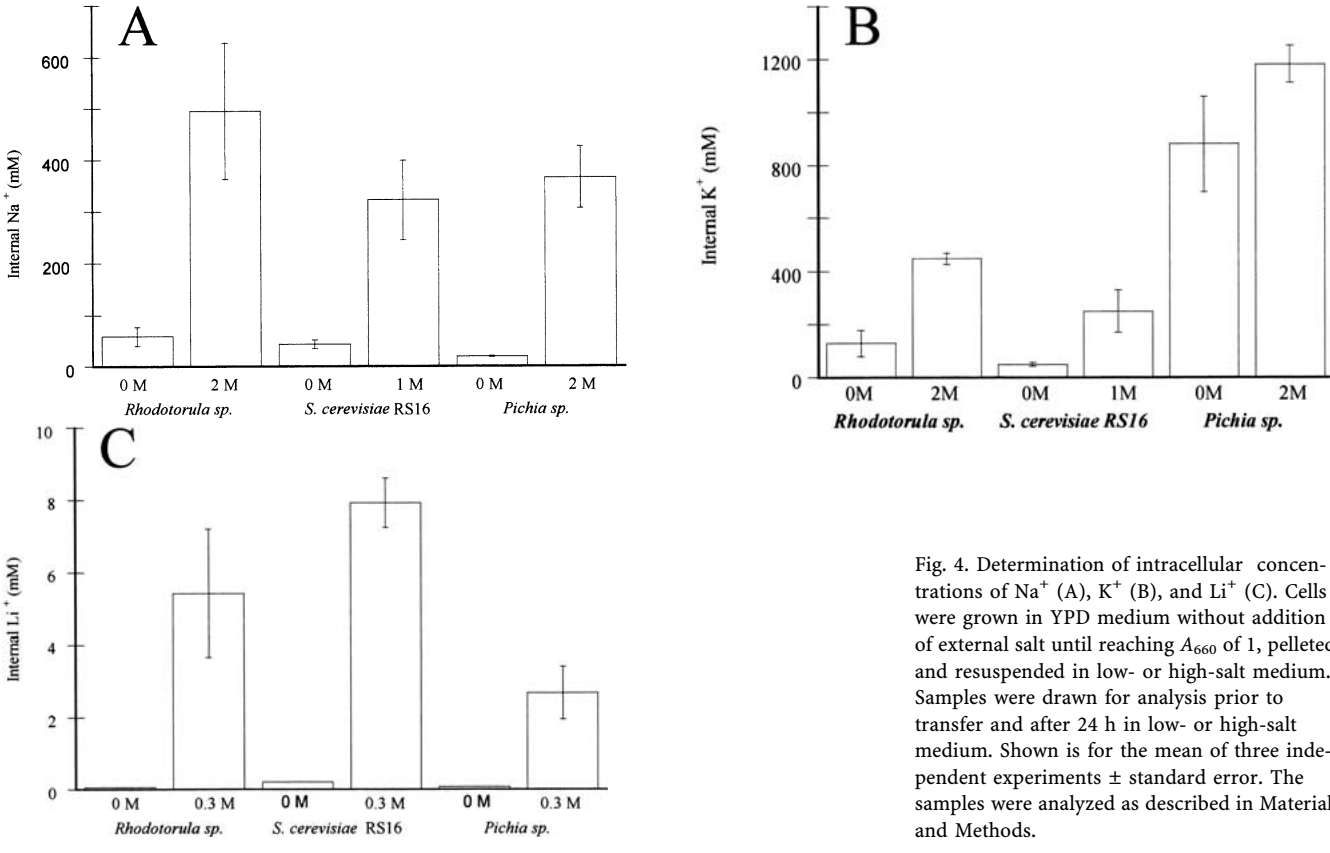

Fig. 4. Determination of intracellular concentrations of Na^+ (A), K^+ (B), and Li^+ (C). Cells were grown in YPD medium without addition of external salt until reaching A_{660} of 1, pelleted, and resuspended in low- or high-salt medium. Samples were drawn for analysis prior to transfer and after 24 h in low- or high-salt medium. Shown is for the mean of three independent experiments ± standard error. The samples were analyzed as described in Materials and Methods.

dium, giving them a clear advantage upon a shift to high-salt medium (Table 1). There was no significant difference between the strains with regard to other solutes upon shift to high-salt medium (Fig. 5).

Using the extraction method described by Band et al. [2] osmolytes were extracted as low molecular weight metabolites (LMWM) in the aqueous phase after transfer to high salt medium. There was a substantial increase in LMWM during the first 6 h in *R. mucilaginosa* and *S. cerevisiae* (Fig. 6). This was maintained after 24 h only by *R. mucilaginosa*. In *P. guilliermondii* and *S. cerevisiae* the levels of LMWM dropped as glycerol concentration increased 24 h after transfer to high salt (Fig. 6). In *R. mucilaginosa* glycerol concentration did not increase above 2-fold while intracellular levels of the LMWM continued to increase. Accumulation of polyols at significant concentrations was not detected by NMR analysis upon shift to high salt but an almost 2-fold increase in trehalose and a 3.5-fold increase of glycine–betaine were observed in *R. mucilaginosa* (Table 1).

Table 1. ^{1}H-NMR quantified solutes (mM) from ethanolic extracts of cells grown in 0 M NaCl and in high NaCl medium[a]

	P. guilliermondii (0 M)	*P. guilliermondii* (2 M)	*R. mucilaginosa* (0 M)	*R. mucilaginosa* (2 M)	*S. cerevisiae* (0 M)	*S. cerevisiae* (1 M)
Glycerol	259.95 ± 55.1	1442.7 ± 176.71	535.00 ± 156.98	1012.5 ± 177.28	17.55 ± 3.90	658.02 ± 241.95
Glycine–betaine	52.36 ± 11.76	161.60 ± 21.49	14.81 ± 5.77	56.10 ± 5.80	49.54 ± 37.40	101.34 ± 21.49
Betaine II[b]	5.40 ± 2.25	0.20 ± 0.35	14.80 ± 1.73	5.23 ± 1.42	0.56 ± 0.49	2.95 ± 0.91
Betaine III[b]	27.90 ± 8.90	32.90 ± 17.10	26.90 ± 15.30	28.55 ± 16.53	18.97 ± 1.80	17.04 ± 1.33
Betaine IV[b]	12.30 ± 3.03	14.93 ± 1.60	5.00 ± 1.10	8.03 ± 1.50	9.70 ± 1.70	15.10 ± 5.20
Trehalose	0	0	75.69 ± 13.35	134.25 ± 29.82	0	0

[a] 3 independent treatments ± standard error.
[b] Different betaine moieties.

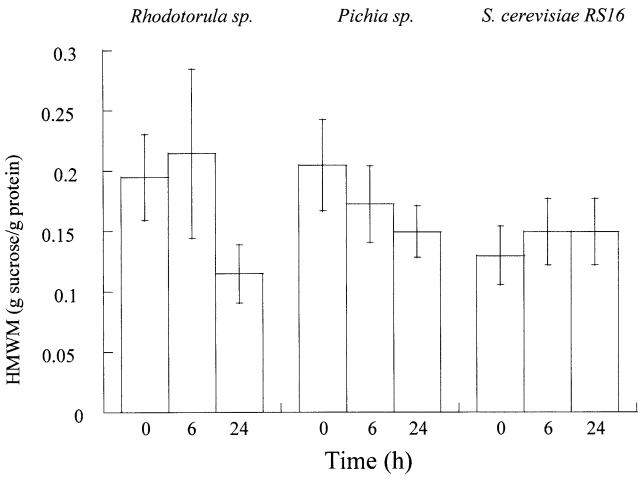

Fig. 5. High molecular weight metabolite determination of cells transferred to high-NaCl medium, based on method by Band et al. [2]. Results, in gram equivalents of sucrose, are the mean of three independent experiments ± standard error.

These observations provide an explanation why the evaporation pond yeast are more tolerant to high salt than *S. cerevisiae*. *P. guilliermondii* is more tolerant to Na^+ than either *R. mucilaginosa* or *S. cerevisiae*. Both *R. mucilaginosa* and *P. guilliermondii* accumulate high concentrations of internal K^+ after shift to high osmotic medium and both strains maintained a high concentration of glycerol in low osmotic media (Table 1), preventing a rapid dehydration of the cells upon instant exposure to a high osmotic environment as is typical in the evaporation ponds. *R. mucilaginosa* accumulated high concentrations of LMWM in response to high-salt medium.

Carbon Source Utilization

A screen for potential carbon sources in the evaporation ponds utilized by these yeast was carried by a tetrazolium salt-based assay. This assay was used previously in tests for susceptibility of yeast to antifungal agents [18].

P. guilliermondii showed moderate to strong MTT reduction in presence of a wide range of carbon sources, including benzoic acid, bisphenol A, anthracene, methanol, bromobenzoic acid, 2-fluorophenol, and raw wastewater. *R. mucilaginosa* showed strong MTT reduction in presence of anthracene, phenanthrene, and benzyl alcohol, and weak to moderate MTT reduction in the presence of phenol, benzoic acid, methanol, and raw wastewater (Table 2).

Previous work demonstrated prevalence of a *Rhodotorula* strain in highly polluted sites [15, 26] and the capacity of a *Rhodotorula* strain to degrade various aromatic compounds such as phenol [26, 21, 22, 23] and phenanthrene [26]. However our *R. mucilaginosa* isolate utilized methanol as an electron donor for respiration; whereas methanol is not known as an energy source for

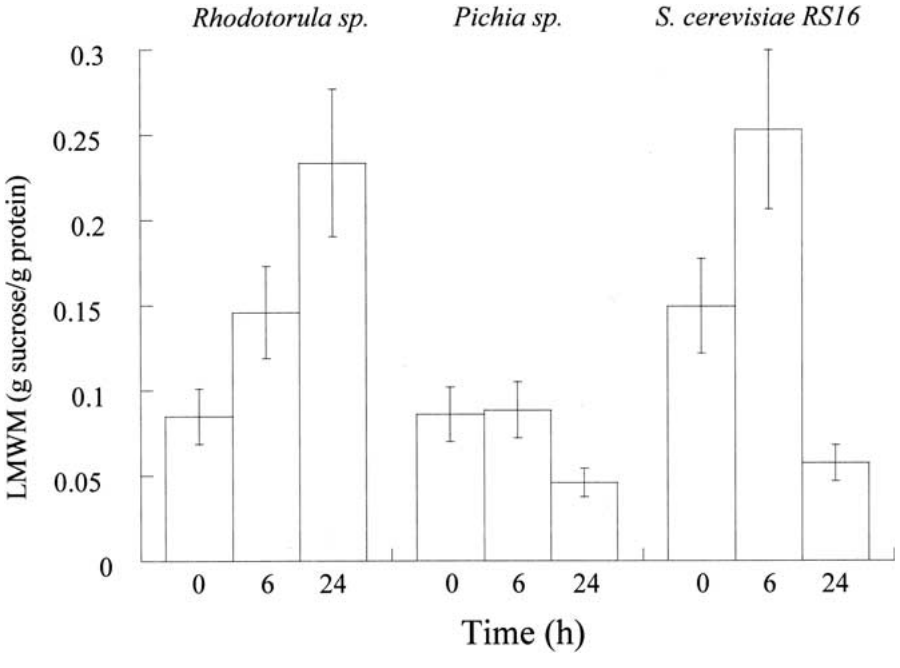

Fig. 6. Low molecular weight metabolite determination of cells transferred to high-NaCl medium, based on method by Band et al. [2]. Shown is the mean of three independent experiments ± standard error.

Table 2. MTT reduction to formazan in the presence of various carbon sources[a] by *R. mucilaginosa, P. guilliermondii* and *S. cerevisiae* strain to RS16

Carbon source	*R. mucilaginosa*	*P. guilliermondii*	*S. cerevisiae* RS16
Bromoacetic acid	85.4 ± 10.9	66.8 ± 8.1[c]	100.8 ± 8.4
Xylene	89.6 ± 2.1	109.0 ± 6.3	120.7 ± 22.9[c]
Phenol	140.0 ± 2.7	119.4 ± 23.9[c]	226.0 ± 27.3[c]
Benzoic acid	227.2 ± 18.6	148.9 ± 16.7[c]	174.3 ± 14.0
Bisphenol A	91.1 ± 2.0	158.8 ± 17.4[c]	135.9 ± 11.2
Anthracene	746.3 ± 100.0***	194.5 ± 22.4[c]	107.5 ± 8.5
Phenanthrene	704.6 ± 19.0***	120.9 ± 16.2[c]	138.6 ± 18.0[c]
o-Methylnaphthalene	66.3 ± 6.8	51.6 ± 5.1	46.8 ± 3.7
Petroleum ether	89.0 ± 8.3	210.1 ± 12.8	25.4 ± 5.5
Methanol	158.2 ± 6.4[c]	167.4 ± 16.4	128.2 ± 13.8[c]
Bromobenzoic acid	85.3 ± 3.2	198.8 ± 11.5	164.9 ± 14.8
Toluene	141.3 ± 12.1	125.9 ± 16.9[c]	160.1 ± 38.0[c]
Glucose	698.1 ± 20.0***	641.1 ± 38.2***	410.6 ± 4.4***
Bromobenzene	144.5 ± 2.7[c]	87.5 ± 16.4[c]	130.8 ± 10.2
Benzyl alcohol	408.1 ± 65.5[b]***	119.1 ± 17.8	352.0 ± 36.1[c]***
Naphthalene	118.3 ± 6.4[c]	104.5 ± 6.2	77.1 ± 13.0[c]
2-Fluorophenol	106.2 ± 5.0	151.3 ± 8.7	129.5 ± 10.0
Evaporation pond water	205 ± 75.5[c]	478.0 ± 130.1[c]***	137.5 ± 34.4[c]

[a] All carbon sources tested, except for evaporation pond water, were tested at the concentration of 100 ppm.
[b] Results from samples withdrawn after 2 days exposure to tested carbon source.
[c] Results from samples withdrawn after 3 days exposure to tested carbon source.
***Significant results.
All other samples were withdrawn after 24 h exposure. All numbers shown are percentages relative to the control (no carbon source added).

Rhodotorula sp. The capacity of this *Rhodotorula* strain to utilize methanol might be a result of gene transfer between the *Pichia* and *Rhodotorula* strains.

Acknowledgments

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